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ALDEHYDE TREATMENT OF CYANIDE POISONING IN MICE

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Eight aldehydes have been tested as therapeutic compounds for cyanide poisoning in mice. The most effective are glyoxal trimer and glyceraldehyde. These increase the LD50 2.5 to 3 times. No correlation was found between activation of the carbonyl group resulting from nearby electron-withdrawing substituents and therapeutic value.

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SUMMARY

Eight aldehydes have been tested as therapeutic compounds for cyanide poisoning in mice. The most effective are glyoxal trimer and glyceraldehyde. These increase the LD50 2.5 to 3 times. No correlation was found between activation of the carbonyl group, resulting from nearby electron-withdrawing substituents, and therapeutic value.

PREFACE

The work described in this report was authorized under Project/Task 1W662710AD2502, Medical Defense Against Chemical Agents; Prophylaxis and Therapy for Lethal Agents. This work was started in September 1973 and completed in October 1973. The experimental data are contained in notebook MN 2543.

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care" as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences–National Research Council.

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CONTENTS

ALDEHYDE TREATMENT OF CYANIDE POISONING IN MICE

I. INTRODUCTION.

Most treatments of cyanide poisoning involve either or both of two approaches:[1] conversion to nontoxic thiocyanate by enzymatic reaction with thiosulfate or tetrathionate in the presence of endogenous rhodanase or its sequestration by binding compounds having a very high affinity for the cyanide ion. The latter includes metal salts and complexes and a limited number of organic compounds; i.e., cobalt EDTA,* methemoglobin, and carbonyl compounds.

For the second approach, carbonyl compound binding would be preferred because these compounds are generally of low toxicity. However, early studies gave poor *in vivo* results.[1]

Recently, Cittadini and coworkers[2] have reported that pyruvate provides modest therapeutic benefit in cyanide poisoning in mice. The cyanide ion is a powerful nucleophilic reagent. It forms very stable cyanohydrins with aldehydes containing electron-withdrawing groups. This point seems to have been overlooked by earlier workers. It provided the rationale for this study.

We have chosen mice for this preliminary screen. At only two animals per dose level, the results are not highly precise. However, they are quite clear cut. Since the better compounds will have to be reexamined in other species, more precise studies with mice seemed unwarranted.

II. EXPERIMENTAL.

Commercially supplied compounds of the highest purity were used as obtained. "Cyanide" was administered as aqueous sodium cyanide. Male albino mice weighing between 18 and 22 grams were obtained from the local colony. The animals were fasted 4 to 6 hours prior to use and were allowed water *ad libitum*.

Test treatment compounds were prepared in 0.9% aqueous sodium chloride (Baxter Laboratories) and adjusted to neutral pH (pHydrion paper) as required.

Toxicity data on treatment compounds (by iv administration) were obtained by the moving averages method of Thompson,[3] calculated according to Weil.[4] Two animals were used at each dosage level, with four or more dosage levels tested per compound. By this procedure, the logarithms of successive dosage levels differ by a constant factor.

The cyanide LD50 values (ip) in the absence and also in the presence of treatment compounds (iv) were determined in the same manner. In each case, a single dose of treatment compound and multiple dosage levels of cyanide were administered. For the treatment compounds, we generally aimed at the maximum sign-free dose; however, many of the compounds were of such low toxicity that these levels were not reached. In these cases, we chose an arbitrary "reasonable" dose of 400 to 500 mg/kg. In the absence of mouse LD50 data, we have, as a matter of convenience, added to table I the reported rat toxicity data for one of the compounds.

*EDTA - ethylene diamine tetraacetic acid.

Table I. Test Results

Test compounds	Intravenous LD50 of therapeutic compound	Therapeutic dose administered	LD50 of sodium cyanide in presence of therapeutic compound (95% confidence limits)	Therapeutic ratio
	mg/kg	mg/kg	mg/kg	
Control	-	-	6.2 (5.0-7.8)	-
Pyruvic acid	-	480	7.6 (6.1-9.5)	1.25
Chloral hydrate	283[a]	200	10.7[a]	1.7
Chloral	400[b] (200-800)	10[c]	<9.5	-
3-Formylpyridine	-	400	<7	-
2-Formyl-l-methylpyridinium iodide	-	400	<7	-
Glyoxal trimer	-	400	17.7 (8.2-38.5)	2.9
Glyoxal (40%, aqueous)	200 (100-400)	100	11.9 (9.5-14.9)	1.9
Glyceraldehyde	(2000)[d]	400	15.9[a]	2.6
Pyruvic aldehyde	141[a]	100	<7	-

[a]Confidence limits cannot be estimated from the data.
[b]Intraperitoneally in peanut oil.
[c]Intravenously, undiluted.
[d]Intraperitoneally, rats; The Toxic Substances List, 1973 Edition, US Department of Health, Education, and Welfare, Public Health Service, National Institute for Occupational Safety and Health, p 476, July 1973.

In a typical run, a suitable volume (approximately 0.1 ml) of aqueous cyanide was injected intraperitoneally followed as soon as possible, but in no case later than 60 seconds, by an iv injection (tail vein) of approximately 0.1 ml of an aqueous solution of the test therapeutic compound.

The animals were observed for a period of at least 4 hours and maintained for 24 hours. All animals that survived for 4 hours recovered completely. Animals that died, died quickly (within 10 min). Recovery rate for the surviving animals varied with the test compound. With most, recovery was rapid.

III. RESULTS.

A. Therapy.

Results are given in tables I and II. The iv LD50 for sodium cyanide in the absence of treatment was calculated to be 6.2 mg/kg, which is somewhat higher than the value of 4.8 mg/kg reported by Cittadini.[2] When pyruvic acid was given after cyanide, the therapeutic ratio (LD50 with treatment/LD50 in absence of treatment) was 1.25 in this work and 1.6 as reported by Cittadini.[2] Among the eight compounds tested, glyoxal trimer is the most effective, raising the LD50 2.9 times. It is striking that glyoxal (monomer, 40% aqueous) is so much more toxic. Also it appears to be less effective as a treatment agent. A similar situation exists with chloral. Here, too, the hydrate is both less toxic and more effective therapeutically than is the free aldehyde. The other promising compound is glyceraldehyde which ranks with glyoxal trimer in therapeutic effectiveness and is second in safety ratio.

B. Miscellaneous Observations.

1. Chloral Hydrate.

In unpoisoned mice, administration of a dose of 100 mg/kg produced a suggestion of hyperactivity. At 200 mg/kg, the mice became sluggish and quiet. Treated mice that received the 9.53 mg/kg dose of cyanide became quite ill, but both recovered completely by the following morning.

2. Chloral.

Chloral hydrates very rapidly upon addition of water and therefore was administered "dry." In the treatment studies, it was administered intravenously, undiluted, using a 10-μl Hamilton syringe. At 10 mg/kg, chloral produced no toxic signs; at 20 mg/kg, two of two animals died. When chloral was administered intraperitoneally, dissolved in peanut oil, the LD50 was 400 mg/kg. At levels of 100 mg/kg and above, the mice became very ill and lost the use of their hind limbs.

3. Glyoxal Trimer.

At 400 mg/kg, mice showed no signs of any sort. Nonsurviving cyanide-poisoned animals that had received glyoxal trimer treatment died more slowly (15 to 20 min) and more "calmly" than did the untreated animals (less than 10 min).

Table II. Experimental Data*

Test compound	Deaths								
	Dose of cyanide (mg/kg) administered intraperitoneally								
	4.0	4.9	6.1	7.6	9.5	11.4	14.2	17.7	22.2
Control	0	0	1	2					
Pyruvic acid			0	2	1	2			
Chloral hydrate			0	0	0	2	2		
Chloral					2**	2**			
3-Formylpyridine			2	2					
2-Formyl-l-methyl-pyridinium iodide			2	2					
Glyoxal trimer				0	0	0	1	1	1
Glyoxal (40%, aqueous)				0	0	1	2		
Glyceraldehyde				0	0	0	0	2	2
Pyruvic aldehyde			0	2	2	2			

*These data provide the basis for the results reported in table I. Two mice were used per test. Each value represents the number that died.

**Deaths were immediate.

Effectiveness of treatment with glyoxal trimer is not highly dose dependent. When the dose of cyanide was 14.2 mg/kg, the results were as follows:

Dose of glyoxal trimer mg/kg	Mortality fraction
50	2/2
100	1/2
200	2/2
400	1/2

4. Glyceraldehyde.

Animals acted perfectly normal after receiving the highest test dose, 800 mg/kg.

IV. DISCUSSION.

Cyanohydrin formation, equation 1, is a reversible reaction. It is the dissociation constant,

$$
\begin{array}{c}R_2\\ |\\ R_1\text{–C=O}\end{array} + HCN \overset{K_D}{\rightleftharpoons} \begin{array}{c}R_2\\ |\\ R_1\text{–C–OH}\\ |\\ CN\end{array}
$$

K_D, which diminishes progressively as the groups R_1 and R_2 are made more electron-withdrawing. The parallel reaction, hydration, equation 2, is similarly affected by electron withdrawal in R_1 and

$$
\begin{array}{c}R_2\\ |\\ R_1\text{–C=O}\end{array} + H_2O \overset{K_H}{\rightleftharpoons} \begin{array}{c}R_2\\ |\\ R_1\text{–C–OH}\\ |\\ OH\end{array}
$$

R_2. In table III, there are data on K_H for two of the compounds. Clearly, the therapeutic value

Table III. Hydration Equilibria*

Compound	K_H** (M)
Pyruvate	18.5
Chloral	3.6×10^{-5}

*Greenzaid, P., Luz, Z., and Samuel, D. J. Amer. Chem. Soc. 89, 749 (1962).

**K_H is the dissociation constant of the hydrate, equation 2.

does not parallel electron-withdrawing qualities. We may speculate on the reasons for the apparent discrepancy.

1. Carbonyl compounds also react with amines, thiols, and other groups found in proteins. It is possible that high reactivity results in loss of therapeutic value through side reactions. In this regard, we find that the free aldehydes, chloral and glyoxal, are considerably more toxic than their aldehyde addition products, chloral hydrate and glyoxal trimer, respectively.

2. In the very rapid reaction with carbonyl groups to form cyanohydrin, it is the cyanide ion which is reactive. Since the pKa of HCN is 9.45, at physiological pH only a very tiny fraction of the cyanide exists as the anion. Hence the overall reaction may be slow.

3. Finally, in aqueous solution, it may be that the critical reaction is between hydrate and HCN. That reaction might be slow (and subject to catalysis) or alternatively may have an unsatisfactory equilibrium constant.

V. CONCLUSION.

The carbonyl compounds show distinct promise in cyanide therapy. The better compounds, such as glyoxal trimer, glyceraldehyde, and chloral hydrate, should be examined in higher animals.

The present study offers no predictive relationships which provide a basis for the choice of improved compounds. Several theoretical models are considered for the failure of therapeutic value to parallel carbonyl reactivity. Further study of the kinetics of the reactions of cyanide with carbonyl compounds in near neutral aqueous media may shed light on this matter and provide a direction for the development of more effective compounds in cyanide therapy.

LITERATURE CITED

1. Paulet, G. L'Intoxication Cyanohydrique et Son Traitement. Masson et Cie, Paris, France. 1960.

2. Cittadini, A., Caprino, L., and Terranova, T. Effect of Pyruvate on the Acute Cyanide Poisoning in Mice. Experientia 15, 943 (1972).

3. Thompson, W. R. Use of Moving Averages and Interpolation to Estimate Median-Effective Dose. Bact. Rev. 11, 115 (1947).

4. Weil, C. S. Tables for Convenient Calculation of Median-Effective Dose (LD50 or ED50) and Instructions in Their Use. Biometrics 8, 249 (1952).